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High immunoexpression of SPANX-C in metastatic uveal melanoma: are SPANX-C-positive trailblazer cells related to the risk of metastasis?

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ABSTRACT

Uveal melanoma is a rare disease but it is the most common primary intraocular malignant tumor in adults with poor late prognosis. About 50% of patients will develop liver metastasis far from the enucleation within 10-15 years. Our study examined SPANX-C expression levels in primary uveal melanoma both with and without metastasis to assess if they can be used to predict metastasis. This study included a total of 55 patients, 28 males and 27 females, with uveal melanoma. A significantly high expression of SPANX-C was seen in 19/23 (82.6%) patients with metastasis, and only in 11/32 (38.5%) patients without metastasis. In conclusion, we found that SPANX-C expression could play a role in tumor progression of uveal melanoma.

Key words

Immunohistochemistry; SPANX-C; Trailblazer cells; Uveal melanoma; Metastasis

INTRODUCTION

Uveal melanoma (UM) is the most common primary intraocular malignant tumor in adults and the second most common melanoma [1] arising from melanocytes not associated with epithelial structures; it has an incidence of 6-7/1,000,000 cases per year and it is considered a rare disease [1] with poor late prognosis; about 50% of patients will develop liver metastasis far from the enucleation within 10-15 years [2]. Because of this strange behavior, which still has to be netter investigated, the authors suggest to search the biological causes to explain late development of liver metastasis of uveal melanoma.

The SPANX multigene family includes SPANX-A1/-A2/-B/-C/-D (Human genome build 36.2). SPANX-A/D genes are made up of two exons separated by an intron of ≈650 bp and were the first SPANX genes described because of their expression in sperm cells [3]. Semiquantitative fluorescent multiplex PCR dosage analysis was carried out to identify 16 classes of SPANX-B and 13 classes of SPANX-C genes [3].

In the literature have been reported immunohistochemical studies on Spanx expression in two melanoma cell lines, VMM150 and VMM5, and in the metastatic melanoma tumor from the VMM150 cell line. These studies revealed the immunostaining at the nuclear periphery, within the nucleoplasm close to the nuclear envelope in the VMM150 cells, while only cytoplasmic staining was observed in the VMM5 melanoma cells. In addition, also the tumor cells from which the VMM150 cell line was derived were positive at the nuclear level [4]. The variable expression of SPANX was also observed in glioblastomas, in which the SPANX-A expression prevails [4]. More Recently, immunohistochemical expression of SPANX has been shown in neoplastic cells of prostatic cancer with a nuclear/cytoplasmic positivity [5], in testicular neoplasms [6], in particular, in seminoma in which the nuclear/cytoplasm staining for SPANX was diffuse and strong, and in embryonal carcinomas in which the expression for Spanx was significantly fainter compared with both normal and seminoma cells [6,7]. The immunohistochemical expression of SPANX was also

studied in melanocytic cells, with no staining in normal melanocytes, an intermediate number of positive cells in benign nevi and a high percentage of positive cell in cutaneous melanoma, with both nuclear and cytoplasm expression [8,9].

In the present study, we examined SPANX-C expression levels in 55 primary uveal melanomas, both with and without metastasis, and we evaluated their association with other high-risk characteristics for metastasis to assess if SPANX-C can be used to predict the behavior of uveal melanoma.

PATIENTS AND METHODS

Patients and Tissue Samples

We performed a retrospective analysis of 55 primary choroidal and/or ciliary body melanomas, treated by primary enucleation at the Eye Clinic, University of Catania, during the eight years up to October 2017. Because of the retrospective nature of the study, no written informed consent from patients was obtained. The research protocols were approved by the Local Medical Ethics Committee (University of Catania) and conformed to the ethical guidelines of the Declaration of Helsinki. Enucleations were performed in cases of tumors not suitable for radiotherapy procedures, such as plaque brachytherapy or proton beam radiotherapy.

The patients included 28 men and 27 women; the median age was 67 years (range 29-85). All cases of uveal melanomas were evaluated for size and location through ophthalmoscopy and A and B scan ultrasonography and presence of metastasis through physical examination, liver ultrasound and total body computed tomography. Median follow up period was 60 months (range 8-138 months). Forty melanomas involved only the choroid (72.7%), while 15 (27.3%) involved both the choroid and the ciliary body; only one case showed extra scleral involvement during surgery, which was

histologically confirmed. Histologically, 11 (20%) were spindle cell melanomas, 16 (29.2%) epithelioid cell melanomas, while 28 (50.8%) were mixed epithelioid and spindle cell melanomas.

According to the TNM classification, pathological T stage of uveal melanomas with metastasis was: pT1a in 1 patient (4.3%), pT2a in 9 patients (39.1%), pT2b in 2 patients (8.7%), pT2d in 1 patient (4.3%), pT3a in 4 patients (17.4%), pT3b in 5 patients (21.7%) and pT4a in 1 patient (4.3%); in the patients with uveal melanomas without metastasis pathological T stage was: pT1a in 6 patients (18.7%), pT1b in 1 patient (3.1%), pT2a in 12 patients (37.5%), pT2b in 6 patients (18.7%), pT3a in 4 patients (12.5%) and pT4b in 3 patients (9.4%).

Considering the 32 patients with primary uveal melanoma without metastasis, 17 were males and 15 females; the median age was 64 years (range 29-84).

Out of the 23 patients with primary uveal melanoma with metastasis, 11 were males and 12 females; median age was 72 years (range 50-85); 13 of the 23 patients died during follow-up from disease progression (tables A,B).

Formalin-fixed and paraffin-embedded tissue specimens were obtained at the Pathologic Anatomy Section, Department G.F. Ingrassia, from section of Anatomic Pathology, GF Ingrassia Department of Medical, Surgical, and Advanced Technologies, University of Catania. The following exclusion criteria were chosen: 1) paraffin blocks containing the tumor could not be used to obtain additional slides for immunohistochemical evaluation, 2) representative tumor tissue was not present in the paraffin blocks, 3) the tumor was totally necrotic, 4) the tumor had been treated previously.

Five sections were obtained from paraffin specimens. Briefly, the slides were dewaxed in xylene, hydrated using graded ethanols and incubated for 30 min in 0.3% H2O2/methanol to quench endogenous peroxidase activity, then rinsed for 20 min with phosphate-buffered saline (PBS; Bio-Optica, Milan, Italy). The sections were heated (5 min × 3) in capped polypropylene slide-holders with citrate buffer (10 mM citric acid, 0.05% Tween 20, pH 6.0; Bio-Optica, Milan, Italy), using a

microwave oven (750 W) to unmask antigenic sites. To reduce the commonly seen non-specific immunoreactivity due to endogenous biotin, sections were pretreated with 10 mg/mL of ovalbumin in PBS followed by 0.2% biotin in PBS, each for 15 min at room temperature. Then, the sections were incubated overnight at 4 °C with rabbit polyclonal anti-SPANX-C antibody (ab1997; Abcam, Cambridge, UK), diluted 1:500 in PBS (Sigma, Milan, Italy). The secondary biotinylated anti-rabbit antibody was applied for 30 min at room temperature, followed by the avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA) for a further 30 min at room temperature. The immunoreaction was visualized by incubating the sections for 4 min in a 0.1% 3,3'-diaminobenzidine (DAB) and 0.02% hydrogen peroxide solution (DAB substrate kit, Vector Laboratories, CA, USA). The sections were lightly counterstained with Mayer's hematoxylin (Histolab Products AB, Göteborg, Sweden) mounted in GVA mountant (Zymed Laboratories, San Francisco, CA, USA) and observed with a Zeiss Axioplan light microscope (Carl Zeiss, Oberkochen, Germany).

SPANX-C staining was identified as either negative or positive. Immunohistochemical expression was assessed as positive when brown chromogen was seen in the nucleus, cytoplasm or cellular membrane. Normal testicular tissue was considered as positive control to evaluate the specific reaction of primary antibodies. Negative controls, involving the omission of primary antibody, were included.

Stain intensity and proportion of immunopositive cells was assessed by light microscopy. Intensity of staining (IS) was graded on a scale of 0–3, according to the following assessment: no detectable staining = 0, weak staining = 1, moderate staining = 2, strong staining = 3, as described previously [10]. The percentage of SPANX-C immunopositive cells (Extent Score, ES) was scored in five categories: <5% (0); 5–30% (+); 31–50% (++); 51–75% (+++), and >75% (++++). Counting was performed at 200× magnification. Staining intensity was multiplied by the percentage of positive

cells to obtain the intensity reactivity score (IRS); IRS <6 was considered Low expression (L-IRS), IRS >6 was considered High expression (H-IRS).

Immunostained slides were separately evaluated by three pathologists (RC, LP and LS), who were blinded to patient identity, clinical status and group identification.

Statistical analysis

Non-parametric comparison of the median values of all parameters in patients without metastasis and with metastasis was performed by Kolmogorov-Smirnov test or Fisher's exact test. Agreement among observers was tested by Cohen K. Considering the median of the values of SPANX-C detected in all patients, each case was classified into two categories (high and low) that expressed SPANX-C higher/equal or lower than the median value. Univariate and multivariate analyses were based on a Cox proportional hazards regression model (time free from metastasis as outcome); this model included gender, age, melanoma location (choroid or ciliary body), cell type (epithelioid, spindle or mixed), extra-scleral extension, pathological T stage, DFS and value of SPANX-C.

All predictors that had a P value <0.15 (cut off) in the univariate analysis were included in the multivariate analysis. Survival analysis according to SPANX-C expression levels was performed by Kaplan-Meyer test; survival rates were compared by log-rank (Mantel-Cox) test. P values < 0.05 were considered as statistically significant.

RESULTS

Immunohistochemistry showed only SPANX-C cytoplasmic staining; SPANX-C was negative in non-tumor ocular tissue. Inter-observer agreement was excellent (kappa = 0.944).

In the whole group (n=55) the median SPANX-C value was 4. SPANX-C expression was high in 23 (41.8%) melanomas, and low in 32 (58.2%) melanomas.

In 32 primary uveal melanomas without metastasis, SPANX-C IS was strong/moderate in 11 cases (34.4%) and weak in 6 cases (18.8%). Fifteen cases (46.9%) were completely negative (Fig. A); ES was >50% in 10 cases (31.3%), variable between 5-30% in 7 cases (21.9%). Only 9/32 cases (28.1%) showed H-IRS, while the remaining 23 cases showed L-IRS (71.9%). (Fisher's exact test, p=0.009, table D). In 23 primary uveal melanomas with metastasis, SPANX-C IS was strong/moderate in 19 cases (82.6%) and weak in 3 cases (13%). Only 1 case (4.3%) was completely negative (Fig. B). ES was >75% in 12 cases (52.2%), >50% in 3 cases (13%), 30-50% in 6 cases (26.1%), < 30% in 1 case (4.3%). 14/23 cases (60.8%) showed H-IRS, while only 9 cases (39.2%) had L-IRS (Fisher's exact test, p=0.009, table D).

No significant statistical difference was found between primary uveal melanomas with and without metastasis and histological subtype (p=0.762). Instead, a significant statistical correlation was found among all the patients, disease free survival (p<0.001) and SPANX-C expression (p<0.001) (table C).

3.3 Correlations between SPANX expression and clinic-pathological factors in uveal melanomas

Factors related to the presence of metastasis at univariate analysis on a Cox proportional hazards regression model were: tumor diameter greater (p=0.057), pT stage (p=0.072), cell type (p=0.005), DFS(p=) Thickness (p=), diameter (p=) and SPANX level (p=0.001); at multivariate analysis only SPANX level (p=0.004) and histologic type (p=0.003) were significant.

Comparing histological type and SPANX expression, no correlation was found (Spearman).

Figure C shows the results of the Kaplan–Meier survival analyses in patients with uveal melanomas with low and high SPANX expression. The survival times free from metastasis (SE, with 95% CI) estimated were respectively: 103.5 (5.0) (CI: 93.8 to 113.3) and 73.6 (12.0) (CI: 50.1 to 97.2).

The log-rank test showed a significant difference between the two groups as regards SPANX expression (p=0.001).

DISCUSSION

Uveal melanoma could be considered an ambiguous neoplasia, considering its biological behavior: regardless of histological subtype and initial stage, uveal melanoma shows liver metastasis also after 10-15 years follow-up.

In the last few years, many papers, reporting the unexplained behavior of uveal melanoma, have compared clinical data and genetic factors, clinical data and protein expression, but to date none has resolved the question.

Previously, we reported the prognostic role of ADAM10, RKIP and pRKIP [10,11], but these proteins did not explain the strange behavior of uveal melanoma, thus they may be considered only potential prognostic markers.

Salemi et al. studied the SPANX gene family and identified the Xq27 region as responsible for genomic rearrangement of deletions, duplication and/or segmental inversions associated with tumor growth [9]. Nuclear and/or cytoplasmic SPANX-C expression has been showed in cutaneous melanoma [9], testicular embryonal carcinoma [7], prostatic adenocarcinoma [5] and glioblastoma [4]. Nevertheless, the role of SPANX in tumor progression of all these tumors is not well understood.

Maine [12] studied SPANX gene expression in a subpopulation of breast cancer cells, defined as "trailblazer cells", able to migrate towards the lung; it has been suggested that tumor cellular phenotypes exhibiting SPANX gene expression could be able to spontaneously migrate far from the primary tumor, throughout the vascular channels, and modify the extracellular matrix in distant organs. Considering the analogy of behavior between lung metastasis from breast cancer and liver metastasis from uveal melanoma, we assessed SPANX-C expression in primary uveal melanomas. In primary uveal melanoma with metastasis we found mainly moderate/strong cytoplasmic

SPANX-C expression (82.6%) and H-IRS (60.8%). However, primary uveal melanomas without metastasis have shown mainly negative expression (46.9%) and L-IRS (71.9%). Moreover, comparing SPANX-C expression in cutaneous melanoma and uveal melanoma, we found a different distribution of the protein, mainly nuclear in cutaneous sites and cytoplasmic in uveal sites, confirming the different biological behavior of the same tumor phenotype.

Considering the above results, we investigated a potential relationship between SPANX-C expression and tumor progression in uveal melanoma.

It is well known that the SPANX gene has no particular enzymatic functions, but that its interaction with other proteins gives it particular characteristics necessary for invasive behavior; in particular, it has been shown that the interaction between SPANX-C and Lamin A/C is necessary to provide structural stability to the nucleus and proper cellular SPANX-C distribution. SPANX-C overexpression in cancer cells has little influence on primary tumor growth, but may be sufficient to determine an invasive phenotypes in which cells are able to change the morphology of their nucleus, adapt to vessel diameter and exit blood vessels [12].

In vivo studies have shown that SPANX-C underexpression reduced metastatic behavior of primary tumors and the number of potential metastatic cancer cells; moreover, as in vitro studies previously reported [12,13], few trailblazer cells could be necessary to select a subpopulation of aggressive cancer cells, even if lacking the epithelial-mesenchymal transition (EMT) phenotype that is able to develop cellular protrusions into the extra cellular matrix and to autonomously invade metastasize.

CONCLUSION

SPANX gene overexpression could make the tumor less responsive to target therapy against the microenvironment and new therapy preventing the development of trailblazer cells could be investigated. Our results confirm the potential role of SPANX-C in tumor behavior: we suppose that the assessment of SPANX-C expression in liver metastasis may be useful to confirm and explain our data, but unfortunately, at present, we have not the possibility to test SPANX-C expression in primary uveal melanoma and liver metastasis.

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Figure Legends

Fig. A

SPANX-C immunohistochemical expression in uveal melanomas without metastasis.

1) Tumor cells are completely negative; 2) Melanoma cells show a weak positivity for SPANX-C (IS 1); 3 and 4 revealed, respectively, a moderate (IS 2) and strong (IS 3) immunoreactivity.

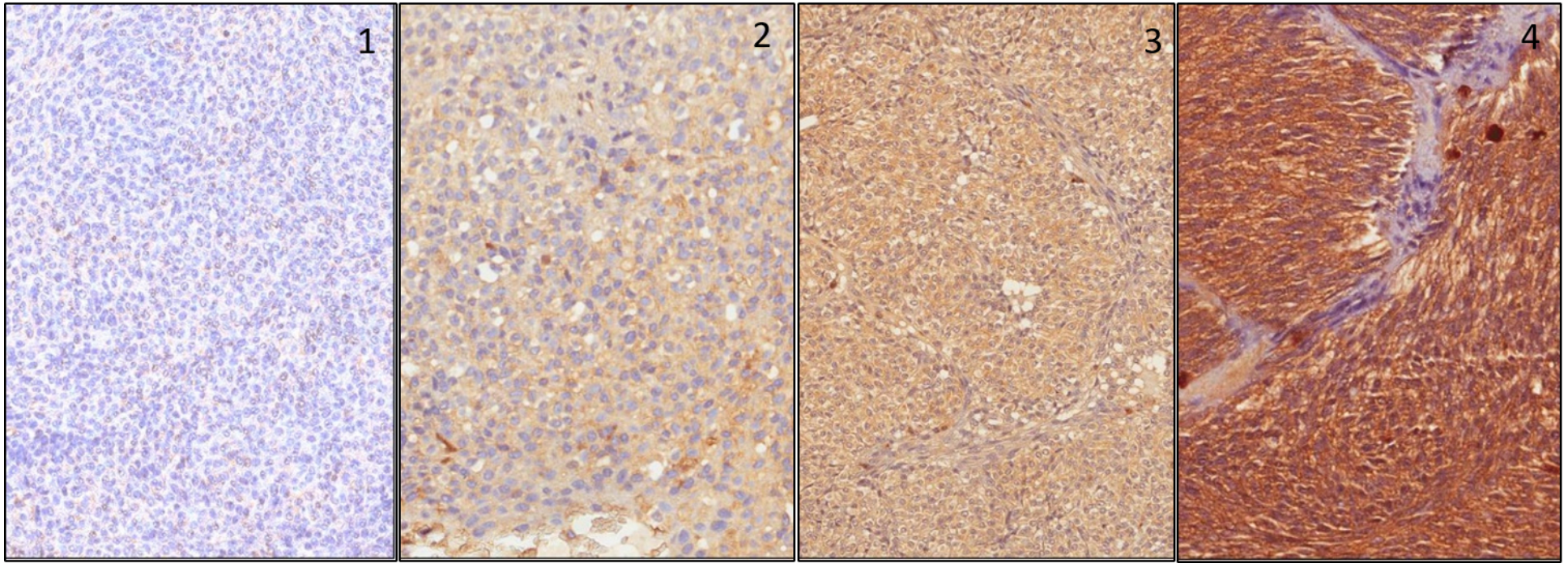
Fig. B

SPANX-C immunohistochemical expression in uveal melanomas with metastasis.

1) Tumor cells are completely negative; 2) Melanoma cells show a weak positivity for SPANX-C (IS 1); 3 and 4 revealed, respectively, a moderate (IS 2) and strong (IS 3) immunoreactivity.

Fig. C

Kaplan–Meier survival analyses in patients with uveal melanomas with low and high SPANX expression.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2
3
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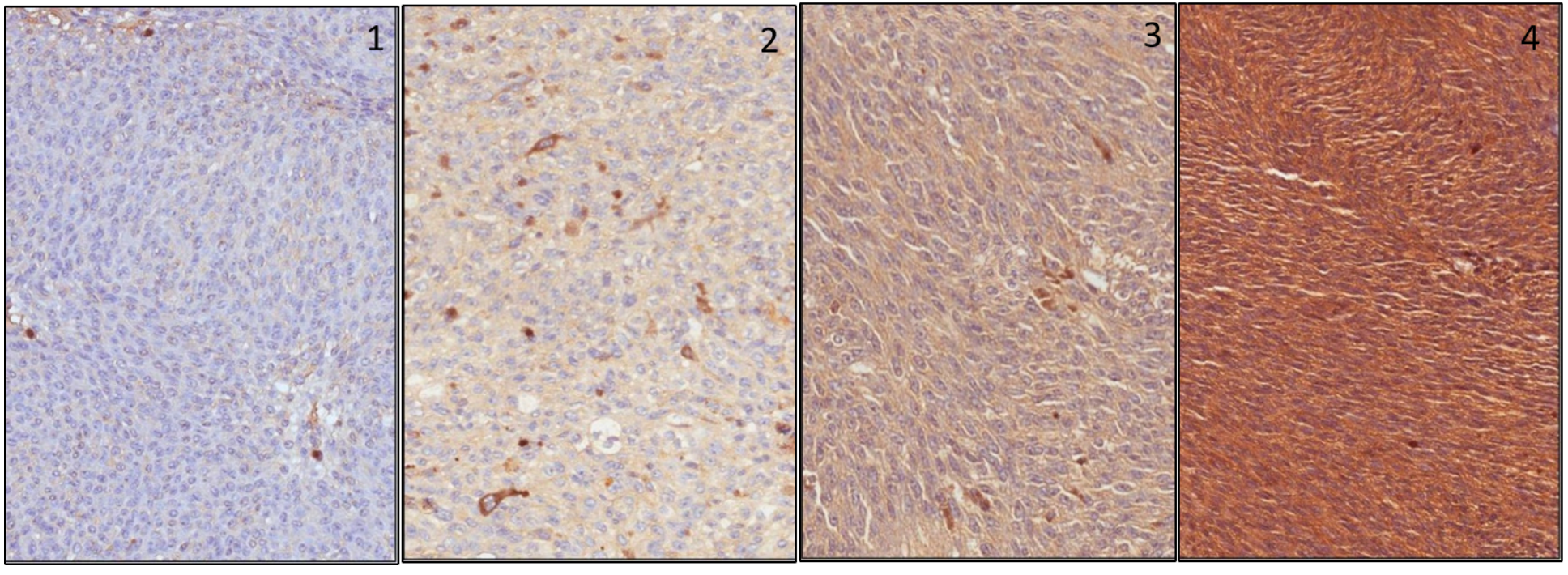
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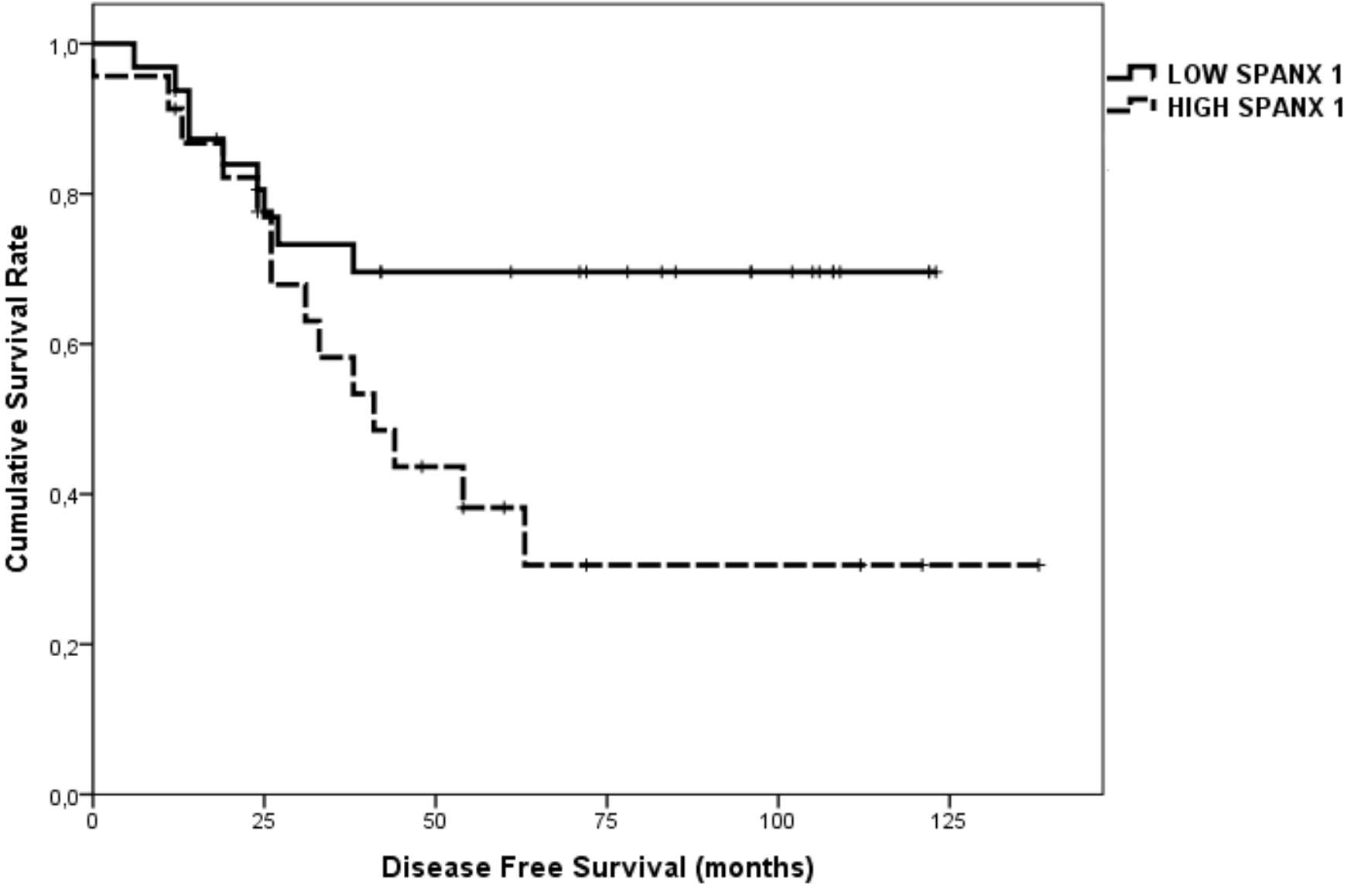
1,0
0,8
0,6
0,4
0,2
0,0
Cumulative Survival Rate
0
25
50
75
100
125
Disease Free Survival (months)
LOW SPANX 1
HIGH SPANX 1

Table A. Demographics, tumour parameters, disease free time, follow-up and SPANX expression in primary uveal melanoma without metastasis.

Sex	Age (yrs)	Location	Thickness (mm)	Largest diameter (mm)	Cell type	Extrascleral extension	Pathological T stage	DFS (months)	Follow-up (months)	SPANX			
										IS	ES	IRS	
F	29	choroid	14,2	16,2	mixed	N	pT2a	138	138	3	4	12	H
F	83	choroid/cil.body	14,84	16,8	mixed	N	pT2b	123	123	0	0	0	L
F	55	choroid	9,8	13,9	spindle	N	pT2a	122	122	0	0	0	L
F	30	choroid/cil.body	12,05	9,2	spindle	N	pT2b	122	122	2	1	2	L
M	74	choroid/cil.body	10,04	16,1	spindle	N	pT2b	121	121	3	4	12	H
M	64	choroid	7,7	11,5	spindle	N	pT1a	112	112	2	4	8	H
F	36	choroid	5,81	12,7	spindle	N	pT1a	109	109	1	1	1	L
F	59	choroid	8,4	16,7	mixed	N	pT2a	108	108	1	1	1	L
M	36	choroid	6,47	9,8	mixed	N	pT1a	108	108	1	3	3	L
M	84	choroid/cil.body	11,9	14,8	mixed	N	pT2b	106	106	0	0	0	L
F	67	choroid	10,42	13.02	mixed	N	pT3a	105	105	1	1	1	L
M	73	choroid	9,7	11,3	mixed	N	pT2a	102	102	1	1	1	L
F	45	choroid	13,7	10,2	mixed	N	pT2a	96	96	1	1	1	L
M	58	choroid	13,1	14,3	mixed	N	pT2a	96	96	0	0	0	L
M	63	choroid	3,3	11,7	spindle	N	pT2a	85	85	0	0	0	L
M	54	choroid	6,32	10	spindle	N	pT2a	83	83	0	0	0	L
F	84	choroid	11,7	17,4	mixed	N	pT3a	78	78	0	0	0	L
M	73	choroid	9,24	17,7	epithelioid	N	pT2a	72	72	0	0	0	L
M	83	choroid	10,62	9,4	epithelioid	N	pT3a	72	72	3	4	12	H
F	71	choroid	3,68	06.04	epithelioid	N	pT1a	71	71	0	0	0	L
M	55	choroid/cil.body	7,5	08.09	epithelioid	N	pT2b	61	61	0	0	0	L
M	52	choroid	9,2	12,1	spindle	N	pT2b	60	60	3	4	12	H
M	46	choroid	8,76	11,3	spindle	N	pT2a	54	54	3	4	12	H
F	76	choroid	8,02	10,7	mixed	N	pT1a	48	48	2	4	8	H
F	63	choroid	10,3	13,7	mixed	N	pT2a	42	42	0	0	0	L
F	41	choroid	5,85	10,3	mixed	N	pT1a	42	42	0	0	0	L
F	55	choroid	3,2	7,6	mixed	N	pT2a	24	24	2	4	8	H
F	74	choroid	8,6	10,2	mixed	N	pT4b	24	24	0	0	0	L
M	68	choroid/cil.body	10,1	10,1	epithelioid	N	pT1b	24	24	0	0	0	L
M	74	choroid/cil.body	14,45	17,5	epithelioid	N	pT4b	18	18	0	0	0	L
M	70	choroid/cil.body	16,27	20,8	spindle	N	pT4b	12	12	2	1	2	L

M	66	choroid	9,2	14,1	mixed	N	pT3a	12	12	3	3	9	H

Tab. A Abbreviations: DFS, disease free survival; SPANX; cil.body, ciliary body.

Table B. Demographics, tumour parameters, disease free time, follow-up and SPANX expression in primary uveal melanoma with metastasis.

Sex	Age (yrs)	Location	Thickness (mm)	Largest diameter (mm)	Cell type	Extrascleral extension	Pathological T stage	DFS (months)	Follow-up (months)	SPANX			
										IS	ES	IRS	
F	58	choroid	6,04	17,8	mixed	N	pT2a	63	64 (†)	3	4	12	H
M	69	choroid	7,21	15,8	mixed	N	pT2a	54	81 (†)	3	4	12	H
F	75	choroid/cil.body	15,5	15,3	mixed	N	pT3b	44	62 (†)	3	4	12	H
F	50	choroid	7,36	15,6	epithelioid	N	pT2a	41	81	3	4	12	H
M	62	choroid	13,68	16	mixed	N	pT3a	38	51 (†)	2	3	6	H
F	51	choroid/cil.body	11,4	18,5	mixed	N	pT3b	38	61	2	2	4	L
M	71	choroid	13,14	17,1	epithelioid	N	pT3a	33	34 (†)	2	4	8	H
M	76	choroid/cil.body	11,6	6,5	mixed	N	pT1a	31	39	3	4	12	H
M	72	choroid	10,3	15,4	mixed	N	pT3b	27	35 (†)	1	3	3	L
F	85	choroid/cil.body	7,3	14,7	spindle	Y	pT2d	26	49 (†)	3	3	9	H
M	73	choroid	5,73	11,7	epithelioid	N	pT2a	26	42 (†)	3	4	12	H
F	51	choroid	9,42	19	mixed	N	pT3a	25	39	2	2	4	L
F	74	choroid	5,7	12,1	spindle	N	pT2a	24	37 (†)	3	4	12	H
F	67	choroid	3,49	20	mixed	N	pT4a	24	31 (†)	0	0	0	L
M	74	choroid	11,35	10,5	epithelioid	N	pT3a	19	47	2	2	4	L
M	82	choroid	9,7	11	epithelioid	N	pT2a	19	42	3	4	12	H
F	72	choroid	6,7	15,2	epithelioid	N	pT2a	14	28 (†)	2	2	4	L
M	76	choroid	13,7	17,1	mixed	N	pT2a	14	70	1	1	1	L
M	79	choroid	13,91	16,1	epithelioid	N	pT3b	13	38	3	4	12	H
F	66	choroid/cil.body	8,95	12,5	mixed	N	pT2b	12	37 (†)	2	2	4	L
F	60	choroid	8,25	16,5	epithelioid	N	pT2a	11	37 (†)	3	4	12	H
F	57	choroid/cil.body	13,6	19	epithelioid	N	pT2b	6	55	1	2	2	L
M	72	choroid/cil.body	13,3	15,4	mixed	N	pT3b	0	51	3	4	12	H

Tab. B Abbreviations: DFS, disease free survival; SPANX; cil.body, ciliary body. (†) death

Table C. Median (range) of demographics, tumour parameters, disease free time, follow-up, SPANX expression in primary uveal melanoma without and with systemic metastasis.

	Sex m-f	Age (yrs)	Location	Thickness	Largest diameter	Cell type	Extrascleral extension	Pathological T stage	DFS (months)	Follow-up (months)	SPANX
All (n=55)	28-27	67 (29-85)	Choroid 40 Chor/ cil.body 15	9.7 (3.2-16.3)	14.1 (6.4-20.8)	Epith: 15 Spindle: 12 Mixed: 28	No: 54 Yes: 1	pT1a: 7 pT1b:1 pT2a: 21 pT2b: 8 pT2d: 1 pT3a: 8 pT3b: 5 pT4a: 1 pT4b: 3	42 (0-138)	60 (8-138)	4 (0-12)
Metastasis free (n=32)	17-15	64 (29-84)	Choroid 24 Chor/ cil.body 8	9.5 (3.2-16.3)	11.9 (6.4-20.8)	Epith: 6 Spindle: 10 Mixed: 16	No: 32	pT1a: 6 pT1b: 1 pT2a: 12 pT2b: 6 pT3a: 4 pT4b: 3	81 (12-138)	81 (8-138)	1 (0-12)
Metastasis (n=23)	11-12	72 (50-85)	Choroid 16 Chor/ cil body 7	9.7 (3.5-15.5)	15.6 (6.5-20)	Epith: 9 Spindle: 2 Mixed: 12	No: 22 Yes: 1	pT1a: 1 pT2a: 9 pT2b: 2 pT2d: 1 pT3a: 4 pT3b: 5 Pt4b: 1	25 (0-63)	42 13 death (28-81)	9 (0-12)
p (metastasis free vs metastasis)		*0.400**	*0.762 °*	*0.911**	*0.007 **	*0.400 **	*0.418°*	*0.560**	*<0.001**	*0.001 **	*<0.001**

Tab. C

- * Kolmogorov-Smirnov test
- ° Fisher's exact test

Table D. Number of uveal melanoma (with and without metastasis) with low and high SPANX

	Metastasis (n=23)	Metastasis free (n=32)
Low	9 (39.1%) *	23 (71.9%)
High	14 (60.9%)	9 (28.1%)

Tab.D Abbreviations: SPANX.

p (Fisher's exact test)
* p=0.026